"Characterizing the Bacterial Communities in Retail Stores in the United States"

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Abstract

The microorganisms present in retail environments have not been studied in detail despite the fact that these environments represent a potentially important location for exposure. In the present study, HVAC filter dust samples in 13 US retail stores were collected and analyzed via pyrosequencing to characterize the indoor bacterial communities and to explore potential relationships between these communities and building and environmental parameters. Although retail stores contained a diverse bacterial community of 788 unique genera, over half of the nearly 118K sequences were attributed to the Proteobacteria phylum. *Streptophyta*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, and *Acinetobacter* were the most prevalent genera detected. The recovered indoor airborne microbial community was statistically correlated with both human oral and skin microbiota, indicating occupants are important contributors, despite a relatively low occupant density per unit volume in retail stores. Bacteria generally associated with outdoor environments were present in the indoor communities with no obvious association with air exchange rate, even when considering relative abundance. No significant association was observed between the indoor bacterial community recovered and store location, store type, or season. However, predictive functional gene profiling showed significant association between the indoor community and seasons. The microbiome recovered from multiple samples collected months apart from the same building varied significantly indicating that caution is warranted when trying to characterize the bacterial community with a single sampling event.

Practical Implications

Like many other indoor environments, the bacterial community in most retail stores is diverse. The retail microbiome is a mixture of microorganisms from many sources including human oral and human skin-associated bacteria, soil bacteria, and the outdoor environment. Analysis of environment and building parameters showed little association between these parameters and the bacterial community present in a given store. Despite utilizing a long-term, integrated sampling approach, substantial variation was observed in repeated samples over a one year period indicating that multiple samples are required to characterize the bacterial community present in environments such as retail stores.

Key Words: Bacteria, 16S, Pyrosequencing, Retail Stores, HVAC Filter Dust, Human Microbiome

Introduction

The indoor bacterial assemblages in retail environments are essentially unstudied, despite microbial health concerns identified in other indoor environments (Burge, 1990; Bouillard et al. , 2005). Exposure to the retail environment is considerable; retail salespeople and cashiers represent the two largest occupations in the U.S. accounting for nearly 6% of the workforce (BLS, 2012). In addition, 39% of Americans purchase consumer goods each day (BLS, 2011). The number of daily visitors and products of a geographically diverse origin in retail buildings represent a unique environment that is different than other studied indoor environments (Frankel et al. , 2012; Nasir and Colbeck, 2010).

There has been relatively little exploration of the retail microbial community or the factors that affect it. To our knowledge, only one study to date has investigated the indoor bacterial microbiome of retail stores using DNA-based methods (Tringe et al. , 2008), albeit Sanger sequencing. In that study of two retail malls in Singapore, the highest percentage of sequences detected were in the Proteobacteria phylum and approximately 60% of the sequences were classified to the taxonomic order Caulobacterales within the class Alphaproteobacteria. The indoor bacterial community was not the same as that observed in the adjacent soil and water samples, suggesting that the indoor environment provided a unique niche for the microbial community.

Given the scarcity of microbial data in retail environments, there is value in considering results observed in other types of buildings. Outdoor bacterial communities have been shown to influence the indoor community observed in residences (Dunn et al. , 2013) and classrooms (Meadow et al. , 2013; Hospodsky et al. , 2012). Kembel et al. (2013) investigated a hospital room with varying levels of fresh air and concluded that the outdoor ventilation and airflow rate can have an influence on both the diversity and composition of the observed microbial communities. In early DNA-based work exploring the relationships between microbial communities and external factors in nursing homes, Rintala et al. (2008) observed that seasonal variations in the bacterial community in a given building were not as significant as variations between different buildings. More recently, multiple composite samples of the metagenome of the urban environment did reveal seasonal variation in portable filter samples (Be et al., 2015).

To investigate the retail microbiome, we used HVAC filters as samplers. The use of HVAC filter dust as a sample of indoor air has been successfully employed in previous studies (Goyal et al. , 2011; Noris et al. , 2011) and can be paired with a molecular technique like pyrosequencing to provide both survey depth and a long-term integrated sample of the indoor environment. Pyrosequencing has been demonstrated to be statistically accurate in phylogenetic comparisons (Liu et al. , 2007), taxa identification (Liu et al. , 2008), and taxa coverage (Porazinska et al. , 2009; Sogin et al. , 2006) and has been used in several recent investigations of the indoor microbiome (Meadow et al., 2013; Hewitt et al. , 2012; Hospodsky et al., 2012). Sampling HVAC filter dust provides a passive, long-term representation of the biological particles present in the indoor air (Noris et al., 2011; Stanley et al. , 2008; Tringe et al., 2008; Möritz et al. , 2001).

The present study investigates the microbial community detected in HVAC filter dust from 13 retail stores, using pyrosequencing to delineate the microbiome of retail environments. The specific objectives of this research were to (1) characterize the indoor bacterial community in select retail stores, (2) identify the influence of potential sources such as the outdoor environment and humans, and (3) delineate associations between the indoor bacterial microbiome and season, location, store type, and air exchange rate. This study represents the largest known DNA-based study of retail environments with 117,808 sequences, 6,100 unique operational taxonomic units (OTUs), and 788 unique genera recovered from 13 retail stores.

Methods

To characterize the indoor microbial community, 22 samples of HVAC filter dust were collected from thirteen retail stores in central Pennsylvania (PA) and Austin, Texas (TX) with multiple measurements in select stores over seasons. As shown in Table 1, each sample collected was given a unique identifier with store type as the first character (e.g. H = home improvement), the second character is a unique store brand identifier, the third letter denotes location (P=PA or T=TX), and the last character is numerical if that store had multiple sampling events over time. The sampling protocol was devised to integrate with a concurrent study (ASHRAE RP-1596) and address feasibility concerns due to store operations (e.g. sampling not allowed during Christmas retail season).

Filter Placement

New clean HVAC filters (multiple manufacturers, all ASHRAE Standard 52.2-2007 MERV 7-8, 50% removal of 3-10 µm particles) were placed in the air handling units of the retail stores for 30 days. The pore size of the filters was standardized to reflect the characteristics of the filters in actual use in retail stores. Altering the pore size would have changed the particle removal efficiencies of particles in the air (Burton et al. , 2007; Möritz et al., 2001). As with other longitudinal methods, the microbial community in the particles collected on the filters may change but that aspect was beyond the scope of the current study. All of the stores had roof-top air handling units that were primarily unducted. The air handling unit selected for testing in each retail store was located as far as possible from any primary building envelope openings and the outdoor air dampers were closed in all the sites except for four (ScP, SdT, FfP, FfT1). These four sites contained a lower number of air handling units so the air dampers were kept open to allow sufficient ventilation air into the stores which operated as normal during the study period. The outdoor dampers were completely closed but it is possible some leakage could have occurred. The four sites that were required to maintain an open damper were an undetermined mix of indoor and outdoor air. Filters from Pennsylvania were kept at 4°C immediately after removal from the unit and shipped to Texas. Filters retrieved in Texas were stored for one night at 4^{o}C to mimic the shipping conditions of the other filter samples. Such low temperature conditions limit the ability of the microorganisms to reproduce in the filter after removal from the HVAC system (Li and Lin, 2001; Lauber et al. , 2010). DNA extraction was conducted after one-day of shipping or storage. Sterile techniques were used for handling the filters in the field. The effect of filter placement location in the retail stores was investigated and it was determined that the bacterial communities collected from different air handling units at the same store over the same sampling period were generally similar (see Supplemental Information). In addition, a subset of four HVAC filter dust samples were examined using scanning electron microscope which showed structural diversity in the dust matrix collected from different retail sites (Supplemental Figure 10).

Metadata

Metadata was collected for four days immediately prior to installation of the clean HVAC filters. Building volume was calculated based on as-built drawings provided by the retail store management and verified during field visits. Seasons were established based on time of the year and average temperatures reported by the National Oceanic and Atmospheric Administration, National Climatic Data Center for Austin, Texas and State College, Pennsylvania. Air exchange rate was determined by releasing sulfur hexafluoride (SF_6) throughout the store, allowing one hour for mixing, and sampling multiple areas in the store for the next four hours in accordance with ASTM Standard E741 (2011). A summary of the metadata collected in the current study is shown in Table 1. More details on these measurements and other metadata collected can be found in the supplemental information and published elsewhere (Siegel et al. 2013; Zaatari et al. , 2014; Zaatari et al. , 2013; Hoisington et al. , 2013).

Table 1. Sampling Location and Metadata Summary

Location	Store Type	Sample ID	Sampling Period	Season	AER (1/h)	Density (#/100m^2)	Volume (m^3)
PA	Home Improvement	HaP1	15 Jun - 14 Jul 11	Summer	0.21	2.2	93,000
		HaP2	8 Nov - 8 Dec 11	Winter	0.2	2.1	
	Small Grocery	ScP	9 Sep - 13 Oct 11		0.76	12	3,300
	General Merchandise	MbP1	8 Nov - 8 Dec 11	Winter	0.56	3.2	99,000
		MbP2	1 Feb - 1 Mar 12		0.4	3.0	
		MbP3	15 May - 21 Jun 12		0.52	2.9	
		MbP4	27 Jul - 23 Aug 12	Summer	0.51	2.7	
	Electronics	EgP1	21 Feb - 14 Mar 12	Winter	0.68	3.1	20,000
		EgP2	6 Jun - 5 Jul 12	Summer	1.37	3.0	
	Furniture	FfP	20 Mar - 19 Apr 12		0.55	0.4	8,100
	Medium Grocery	GeP	3 Aug - 4 Sep 12		0.87	10	25,000
TX	Home Improvement	HaT	18 Jun - 19 Jul 11		0.3	1.8	92,000
	General Merchandise	MbT1	21 Jul - 23 Aug 11		0.51	3.2	61,000
		MbT2	28 Oct - 28 Nov 11		0.99	3.2	
		MbT3	3 Feb - 5 Mar 12		0.42	3.2	
		MbT4	27 Apr - 27 May 12		0.49	3.2	
	Small Grocery	SdT	4 Aug - 15 Sep 11		0.93	13	5,400
	Medium Grocery	GeT1	16 Sep - 17 Oct 11	Summer	1.07	12	15,000
		GeT2	3 Feb - 5 Mar 12	Winter	1.14	5.8	
	Furniture	FfT1	17 Oct - 17 Nov 11		0.25	1.1	20,000
	Office Supply	OhT	17 May - 19 Jun 12		0.29	2.1	21,000

DNA Extraction

DNA extraction was conducted as described by Noris et al. (2011). Dust was extracted from nine evenly spaced 2.5-cm^2 pieces of each HVAC filter and transferred into a presterilized phosphate buffer solution (PBS; 10 g/L NaCl, 0.25g/L KCl, 1.43 g/L Na_2HPO_4, 0.25 g/L KH_2PO_4, DNA-free water, pH 7.0) in a sterile 50 mL centrifuge tube (Thermo Fisher Scientific

Inc., Waltham MA). The solution was sonicated and vortexed for 10 minutes each. The liquid was passed through a 20 µm pore size filter (Whatman Ltd., Maidstone United Kingdom) as a prefiltration step. The filtered solution was vacuum filtered through a 0.2 µm hydrophobic filter (Millipore, Billerica MA). The filter, 100 µL lysozyme (3mg/mL) and 300 µL phenol-chloroform-isoamyl alcohol (24:24:1) were placed into a bead tube (lysing beads and 750 µL lysing solution) provided in the PowerSoil DNA Isolation Kit (Mo-Bio Laboratories Inc., Carlsbad CA). Cell lyses by multidirectional beating of matrix beads was conducted in the FastPrep-24 homogenizer (MP Biomedicals LLC, Solon OH), following manufacturer recommendations of 30 seconds at 5.0 m/s. All DNA recovered was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham MA) and stored at -20^{o}C.

Pyrosequencing

An aliquot of extracted DNA was amplified and processed via barcoded, multiplexed pyrosequencing (Research and Testing Laboratories Inc., Lubbock TX) on a 454 FLX+ Titanium Genome Sequencer (454 Life Sciences, Branford CT). Primers for amplification covered the first three variable regions of the 16S rRNA gene (V1-V3), 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKCTG-3'). Additional details on pyrosequencing processing including PCR conditions, barcoding method, and bead descriptions are published elsewhere (Dowd et al. , 2008). Comparison to other studies that do not share the same primer sets, as is common in microbiome research because no universal primer has been accepted in the field. One sample, corresponding to site FfT2 (Furniture store in TX), contained a low quantity of reads compared to the other samples and was discarded from further analysis. Reproducibility was checked through blind triplicate sampling of aliquots of the same extracted DNA, revealing nearly identical microbial communities (see

Supplemental Information). In addition, pyrosequencing of extracted DNA from the samples was observed to capture considerably more of the microbial community compared to the traditional culturing methods (see Supplemental Information).

Sequence Processing

Processing of the sequences were conducted in the QIIME 1.8.0 open-source software package (Caporaso et al. , 2010) using the overview tutorial pipeline unless otherwise noted. Initial quality filtering included deleting reads under 250 bp, removal of noise in reads (Quince et al. , 2011), and deleting of chimeric reads (Edgar et al. , 2011). After quality filtering, the mean read lengths were 395 bp and the mean sample depth per sample was 3,167 sequences. Taxonomic identification was determined by alignment based on conserved regions using the basic local alignment search tool (BLAST) algorithm (Altschul et al. , 1997) on a National Center for Biotechnology Information (NCBI) curated nucleotide database. Operational taxonomic units were clustered to 97% similarity using de novo UCLUST (Edgar et al., 2011). Alignment was performed with the PyNAST algorithm (Caporaso et al. , 2009) against the GreenGenes reference dataset, updated 12 Dec 2012 (McDonald et al. , 2012). To limit sequence depth bias (Gihring et al. , 2012), samples were rarefied to 1,200 sequences for analysis based on the minimal sequence count in one sample. In some cases, the rarefaction level was adjusted to provide a more direct comparison of the retail sequence dataset to the datasets collected in other studies. Due to the different number of sequences obtained in the samples, alpha-diversity was determined from OTUs (i.e. observed species) in a rarefied sample (Lundin et al. , 2012). Beta-diversity was calculated using weighted UniFrac, a phylogenetic distance matrix that compares diversity in samples (Lozupone et al. , 2006). Statistical analysis of associations was performed in Stata Version 12 (StataCorp, 2011), QIIME (Caporaso et al., 2010), and STAMP (Parks et al., 2014). Statistical tests included t-test, cluster analysis, mantel test, and principal coordinates analysis (PCoA). Additional details on statistical tests performed and associations can be found in the supplemental information. UniFrac pairwise comparisons were computed using a weighted UniFrac significance based upon 100 Monte Carlo simulations. Predictive functional composition of the functional metagenome using 16S rRNA sequences was conducted using PICRUSt software (Langille et al., 2013) and the resultant genes compressed into KEGG pathways were analyzed in QIIME and STAMP. All sequence data are publically available through the European Bioinformatics Institute (EBI) under accession numbers ERS370920-ERS370941.

Results and Discussion

Characterization of Bacteria in Indoor Retail Environments

A total of thirteen stores in Texas and Pennsylvania were investigated including two stores in all four seasons and three stores in two seasons. In Figure 1, the relative abundances of the 24 unique phyla observed at the retail sites are shown. Proteobacteria was in general the most abundant phylum detected (55% of the sequences). Proteobacteria was also the dominant phylum observed in dust samples recovered from aircraft filters (Korves et al. , 2013) and mall filters (Tringe et al., 2008). The next two most abundant phyla detected in the stores were Actinobacteria (19%) and Firmicutes (17%). The relative abundance of the phyla varied across the retail sites, especially for Proteobacteria and Actinobacteria. The high variability at the phylum level is one indicator that the retail microbiome is diverse. Interestingly, the three most abundant phyla observed in the present study were consistent with the most abundant phyla observed in a residential study by Dunn et al. (2013).

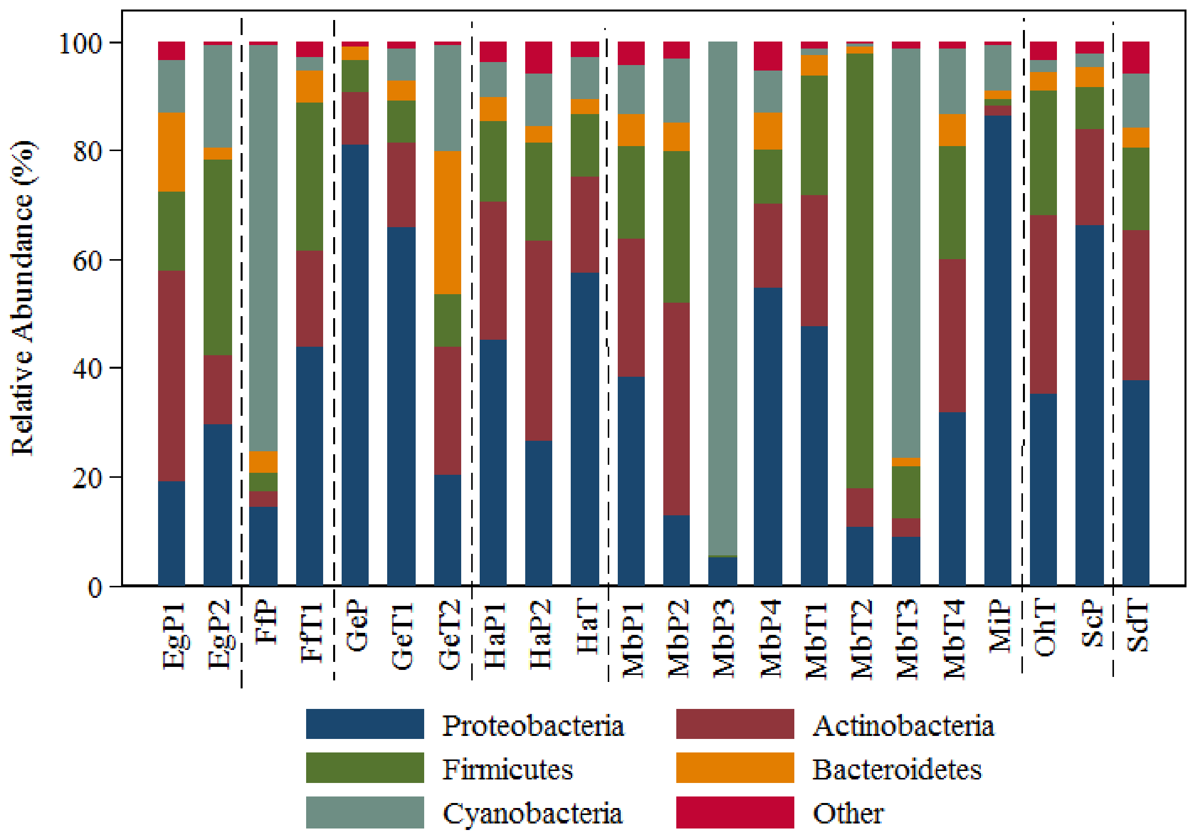

Figure 1. Relative abundance at the phylum taxonomic level for the retail stores grouped by category (dashed lines separate store types; E=electronics, F=furniture, G = medium grocery, H = home improvement, M= general merchandise, O = office supply, S = small grocery).

At the genus level, 788 unique genera (median: 141 unique genera/store, 1st Quartile (Q_1): 85 unique genera/store, 3rd Quartile (Q_3): 174 unique genera/store) were observed in this study with *Methylobacterium* species being the most abundant (20.3%). The dominance of *Methylobacterium* was influenced considerably by the sequences recovered from four sites (FfP, MbP3, MbP4, MbT3) in which this genus represented greater than 70% of the sequences. If the four outlying sites with high *Methylobacterium* abundance are removed, the average relative abundance decreases to 6.8%. *Methylobacterium* is ubiquitous in nature (Gallego et al. , 2005) and has been observed in biofilms on HVAC heat exchangers (Hugenholtz et al. , 1995; Schmidt et al. , 2012), residences (Martin et al. , 2009) and even in the human eye (Dong et al. , 2011). However, classification of the *Methylobacterium* sequences through the Ribosomal Database Project (Cole et al. , 2009) indicates that the *Methylobacterium* detected in this study were actually *Streptophyta* (more specifically from a chloroplast 16S rRNA gene in the Cyanobacteria phylum). The NCBI database used in the present study for the sequence identifications removed all chloroplast 16S rRNA which explains the two different classifications. Chloroplast rRNA has been identified in other dust studies with relative abundances ranging from 10 to 23% (Täubel et al. , 2009; Pakarinen et al. , 2008). The *Streptophyta* classification supports the hypothesis that these sequences were likely from an outdoor source.

The next most abundant genera observed in this study were *Bacillus* (5.8%), *Corynebacterium* (4.6%), *Pseudomonas* (4.0%), and *Acinetobacter* (3.0%) which are all commonly detected in the indoor environment (Täubel et al., 2009; Rintala et al., 2008; Hewitt et al., 2012) (See Supplementary Information for top five most abundant genera at each site). For comparison, in the only study available for the retail environment (Tringe et al., 2008), more than 50% of the sequences belonged to the genera *Brevundimonas* and *Stenotrophomonas*, which in this current study only represented 1.3% of the sequences, highlighting a well-known variability in indoor environments.

The indoor environment in these occupied retail stores yielded 4,770 unique OTUs. Of these OTUs, 71% were unique and observed in only one sample and 70 OTUs were observed in 50% of the samples. The total number of OTUs (median: 380 OTUs, Q_1:182 OTUs, Q_3: 562 OTUs) detected in each samples, correlates with the total number of unique genera identified (Kruskal-Wallis Test, $p<0.0001$). When rarefied to 1,200 sequences, the average number of OTUs per store was 290 which is similar to the diversity recovered at the same rarefaction levels from HVAC filter dust in malls (Tringe et al., 2008) and residential units (Dunn et al., 2013) but approximately half the diversity of floor dust in densely populated classrooms (Hospodsky et al., 2012). The rarefaction curve showed an increase in sampling depth would result in additional unique OTUs (Supplemental Figure 5), a finding that is consistent with other studies (Hospodsky et al., 2012; Yamamoto et al. , 2012).

Predictive functional profiling was conducted using PICRUSt computational software using the 16S rRNA marker gene sequences. The nearest sequenced taxon index (NSTI) quantifies the phylogenetic distance between the given sequences and known referenced genomes. The retail samples NSTI (median: 0.11, Q_1: 0.045, Q_3: 0.18) were higher than the human microbiome index but less than other more diverse samples that have successfully utilized PICRUSt during validation testing (Langille et al., 2013). The KEGG orthologies (KO) were collapsed to tier 1 to 3 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for analysis. Over 52 million gene functions were predicted for the bacterial community recovered from the retail store samples. The predicted tier 2 metabolic pathways with over 1% relative abundance are shown in Figure 2. Overall, the predictive gene functions were similar across the retail samples with the largest variance in tier 1 groupings (coefficient of variance 3.6 – 18%). The KEGG pathway of sporulation was observed at a median relative abundance of 0.15%. The low sporulation abundance may indicate that the microbial community in the HVAC filter dust is representative of the microbiome in the air and the desiccation from the air flowing through the filter did not selectively alter the bacteria community present.

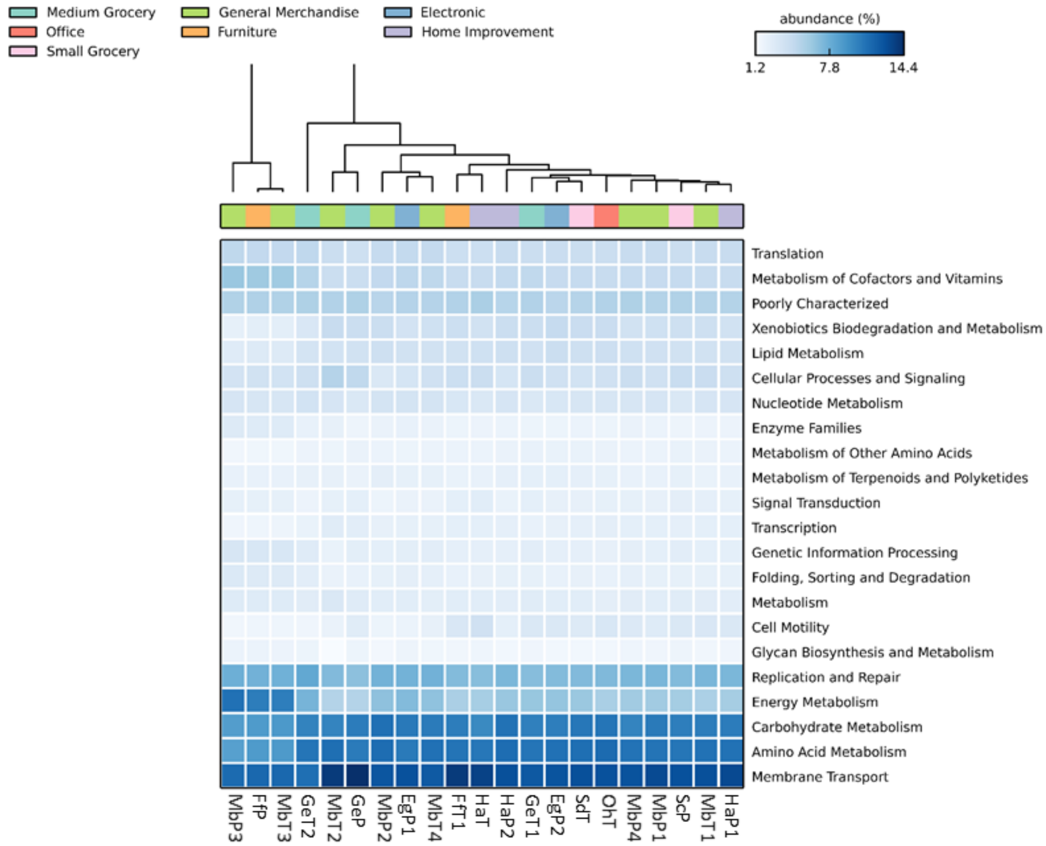

Figure 2. PICRUSt predicted metabolic pathways (Tier 2, showing only over 1% relative abundance).

Microbial Sources

The sources of microorganisms in the indoor environment can be broadly divided into those related to the outdoor environment, to indoor products, and to human occupants. Identifying the influence of human microbiota on the indoor microbiome is non-trivial due to the high variation in microorganisms between individuals (Grice et al. , 2008; Knights et al. , 2011b). One simplified approach to estimate the influence of human-associated bacteria suggested by Hospodsky et al. (2012) is to quantify the relative abundance of selected human indicator bacteria (i.e., Proprionibacterineae, Enterobacteriaceae, Corynebacterineae, *Staphlococcus*, and *Streptococcus*). The median relative abundance of these human-associated taxa in the retail environment was 10% (Q_1: 5%, Q_3: 17%) of the total taxa which is less than the 17-20% reported in the classroom study. The greater abundance of these taxa in the classroom study may be due to the higher density of human occupants in classrooms as compared to retail stores. The average occupancy in the classroom study (measured in number of people per volume of indoor space) was seven times higher than that estimated in the retail stores (see Supplementary Information for full details on occupancy at the test sites). Alternatively, it is plausible that sampling HVAC filter dust that has no direct human contact reduces the abundance of human-associated taxa relative to resuspended dust in a classroom setting or other high occupancy settings. In a study of residential homes, Dunn et al. (2013) used SourceTracker (Knights et al. , 2011a) to determine that 30% of bacteria found on toilet seats and pillowcases in residences were human-associated. In contrast, less than 8% of the taxa recovered from the top of the interior door trim were human-associated in this study. Application of SourceTracker to the present HVAC data set reveals that 17% (Q_1: 10%, Q_3: 35%) of the sequences in the present study were associated with the human microbiome.

Figure 3 presents the principal coordinates analysis results of the microbial assemblages from HVAC filter dust in retail stores as compared to the human associated microbiome, drawn from multiple individuals by Costello et al. (2009). The distance matrices from the bacterial communities recovered at retail sites were significantly correlated to both human oral and skin microbial communities (Adonis Test, $p<0.001$). The same trend was observed when using the unweighted UniFrac method that does not consider relative abundance, thus indicating a relationship exists between the samples based solely on the microorganisms present. Although a connection between human bacteria and indoor microbiome has been reported for residential floor dust (Täubel et al., 2009), it is notable that the same general conclusion can be observed in the retail sector, with an average building in this study of 38,600 m^3 which is approximately seventy-five times that of typical single family homes.

In addition to oral and skin microbiota, communities observed in the retail stores clustered closely to soil microbiota (Figure 3), although the communities were not significantly correlated. Even when the outlying gut samples are removed, the same pattern is observed (Supplemental Figure 6). The weaker association between the filter and the soil bacterial communities may be attributed to differences in aerodynamic particle size and ability to withstand the environmental conditions in the filter. The larger soil particles are not as likely to be aerosolized into retail HVAC inlets located 10-15 meters about the floor. A high relative abundance of Acidobacteria in soil microbiome is the reason for the distance seen between communities in the PCoA plot.

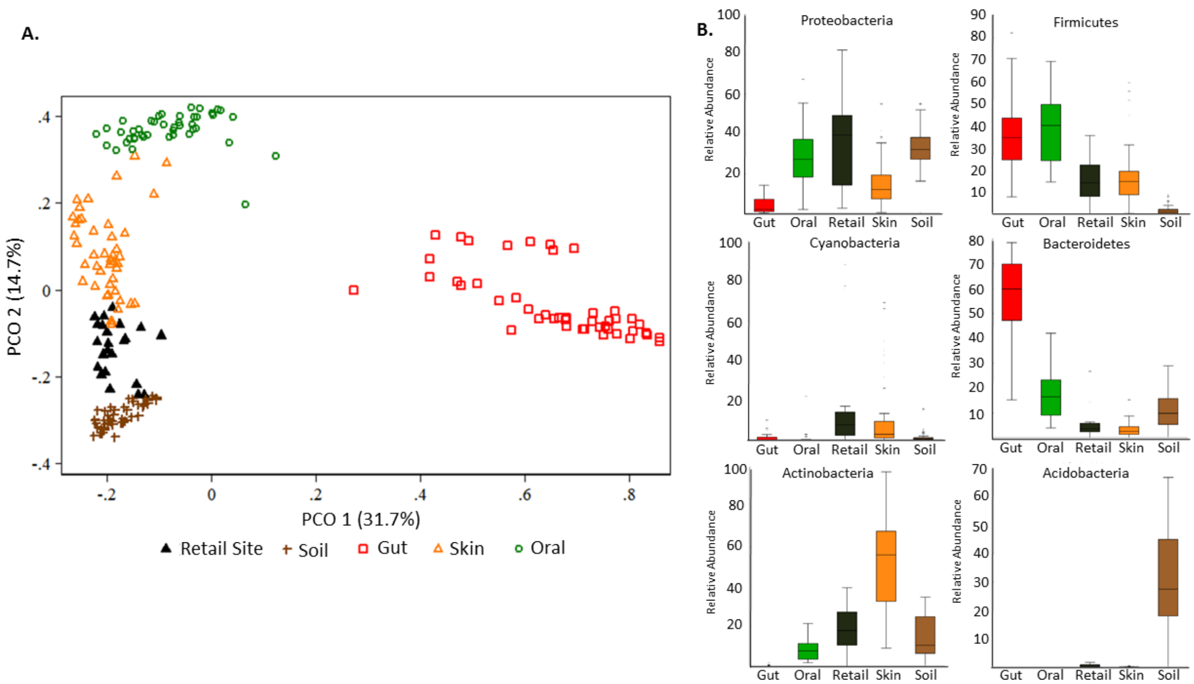

Figure 3. Community comparison between retail site, human (from Costello et al., 2009), and soil microbiomes (from Lauber et al. , 2009). (A) Principal coordinate analysis (PCoA) plot comparing retail sites and others (weighted UniFrac, rarefaction 600 sequences/sample). (B) Scatter box plots showing relative abundance across six phyla

Another potential source for the microbial community recovered in the HVAC filters is the outdoor air. As mentioned above, four sites contained a high percentage of *Streptophyta* (RDP classification). Plastids have been described as evolved from cyanobacteria in Mereschkosky's 1905 article (translated from German to English by (Martin and Kowallik, 1999)). The presence of groceries in the retail sector did not correlate with the relative abundance of *Streptophyta*. Thus, it could be hypothesized that these sequences belonged actually to a chloroplast 16S rRNA gene from outdoor plant materials, which supports to the hypothesis that sites FfP, MbP3, MbT3, and MiP were strongly linked to the outdoor environment. Additional analysis was conducted and PCoA plots (Supplemental Figures 7-9) indicates that the microbiomes recovered from these four retail sites microbiomes are closely related to HVAC filter dust samples collected in other studies (Hospodsky et al., 2012; Kembel et al., 2012) that did not close the outdoor air ducts to limit the amount of outdoor air being filtered.

Influences of Building and Environmental Parameters

The potential influences of season, location, store type, and air exchange rates on the bacterial microbiome were investigated. To analyze seasonal trends, four stores were investigated across two seasons (summer and winter). The types of retail sites selected were: an electronics store (summer: EgP2, winter: EgP1), a medium grocery (summer: GeT1, winter: GeT2), a home improvement store (summer: HaP1, winter: HaP2), and a general merchandise store (summer: MbP2, winter: MbP4). Biological measurements, including number of OTUs, phylum relative abundance, or evolutionary distances, were not found to be correlated with season (see Supplemental Table 1). However, it was observed that the genus Acinetobacter, linked to nosocomial infections (Bergogne-Berezin and Towner, 1996), was the most abundant genera (in relative terms) observed in the summer, with abundances eight-fold higher in the summer than in the winter. Bowers et al. (2011) sampled outdoor air in Midwest cities and found a significant seasonal association between winter and summer in terms of bacterial composition and abundance. The limited association in the current study may suggest that the bacterial community in air conditioned indoor retail environments is influenced by a variety of factors and not just the outdoor environment surrounding the stores.

However, predictive functional genes were found to differ the most as a function of seasons but not as a function of store location, store type, and air exchange rates. Seventy-two tier 3 and 13 tier 2 KEGG pathways (Figure 4) were significantly different between the seasons (ANOVA, $p<0.05$). Cell motility as well as cellular processes and signaling were both higher in the summer when higher temperatures allow the bacterial cells to expend more energy. In addition, more viable cells in the summer may cause an increased need for motility to obtain substrate.

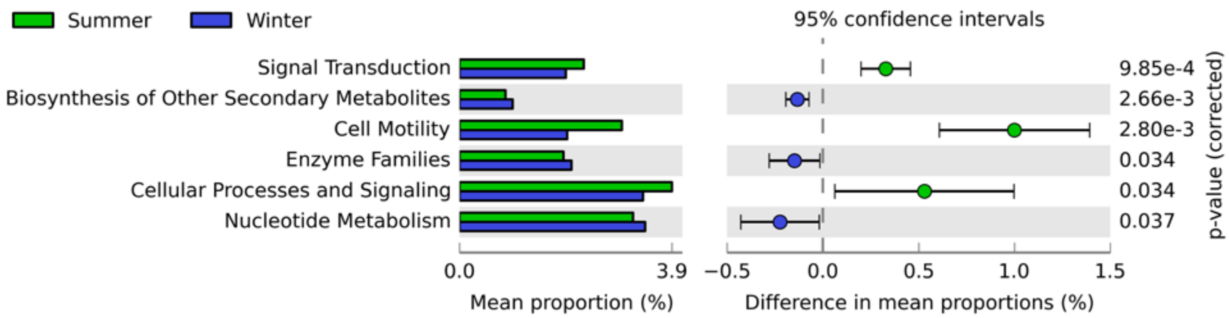

Figure 4. PICRUSt predicted metabolic pathways (Tier 2) with significant differences in relative abundances between summer and winter sampling.

The effect of spatial location was analyzed based upon 12 retail samples in Pennsylvania and 10 retail samples in Texas. Texas retail stores had slightly higher bacterial diversity than the Pennsylvania stores (mean 304 OTU/store vs. 277 OTU/store). At the phylum taxonomic level, Firmicutes sequences were observed at a higher relative abundance in Texas sites (22.6% TX vs. 13.1% PA) at the expense of Proteobacteria sequences (48.9% TX vs. 60.0% PA) however, both phyla comparisons are not statistically significant different. The ability of Firmicutes to form endospores provides them with an evolutionary advantage that may allow them to survive better in the drier climate of Texas. For example, *Bacillus* was observed in 11.2% of the Texas sequences and only 1.2% of the PA sequences. The clustering results for the retail sites are shown in Figure 4 with PA sites in gray and TX sites in black. Despite the higher Firmicutes levels in Texas, the results indicate that location was not a major factor in the bacterial community comparison of the retail stores in this study.

To determine if the type of store affects the microbial community present, the impact of store type on the bacterial community was investigated by comparing the bacteria recovered from stores in Texas and Pennsylvania with identical store brands. Based on this criterion, store categories included in this analysis were general merchandise, medium grocery, small grocery, furniture, and home improvement. Three or fewer samples were collected for each store category with the exception of general merchandise (9 sampling events in 3 stores). Therefore, the results presented are exploratory in nature and may be influenced by other factors that were changing between the sites. With these caveats, the category of small groceries contained the most diverse community, significantly larger than the other store types (t-test, $p<0.0001$). No statistical associations were observed between store categories and bacterial communities at the phylum or genus taxonomic levels (see dendrogram, Supplemental Figure 7 and Supplemental Table 1). Similar results were obtained with the unweighted UniFrac method which does not consider relative abundance data (data not shown) and through predictive function genes analysis (Figure 2). Additionally, Leff and Fierer (2013) observed a high relative abundance of *Enterobacteriaceae* on fruits and vegetables but that bacterial family was not observed at higher levels in grocery stores relative to that observed other store categories (ANOVA, $p=0.46$). It appears that the type of store is not a major influence on the indoor bacterial microbiome but definitive conclusions cannot be reached due to the relatively small number of store types investigated in this study.

Although no direct relationship was observed between the type of retail store and the microbiome recovered from a given store, it was of interest to determine how the microbiome recovered from the retail environments compared to that recovered from other building types including offices and home environments. Office and retail environments are similar in that they housed within commercial office buildings that do not contain living quarters. The results of this comparison (Figure 5) indicate that the office and retail microbiomes were more similar to each other than dust samples collected from residential indoor environments. It should be noted that the residential sample set was sequenced via Illumina while 454 sequencing was used for the office and retail samples. Differences in the sequencing methods employed as well as the type of indoor samples collected could have contributed to the differences observed in the microbiome recovered from the retail, office and home environments

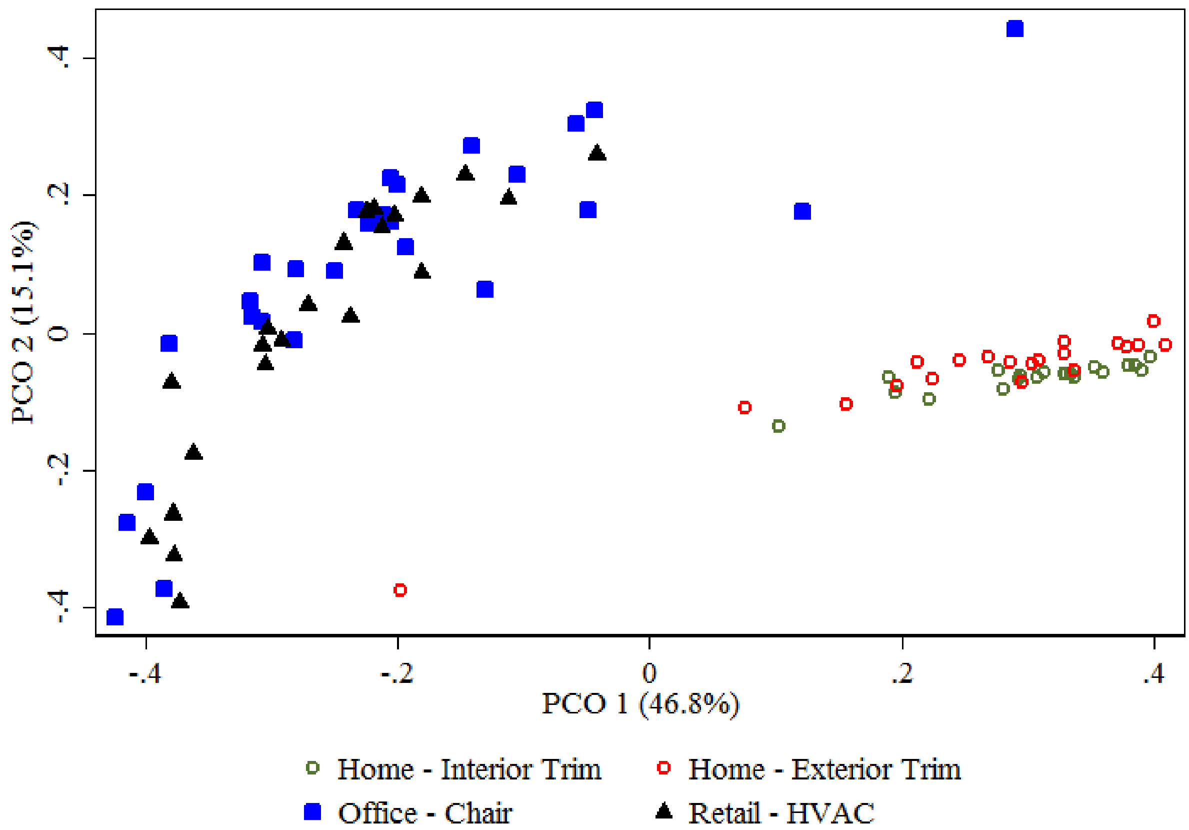

Figure 5. Community comparison between retail site, home interior and exterior trim (from Dunn et al. 2013), and office chairs from (Hewitt et al. 2012)

In our retail sampling, the air exchange rate (AER) was not correlated to the number of observed species, OTUs, or predominant phyla. Several studies in the past have investigated the AER and bacterial concentrations through culture-based methods (Frankel et al., 2012; Kruppa and Rüden, 1996). In these studies, AER and viable bacterial concentrations have been shown to be either negatively correlated (Bartlett et al. , 2004; Frankel et al., 2012) or not correlated

(Kruppa and Rüden, 1996; Wu et al. , 2005). When the dampers in an HVAC system are open, the indoor air becomes more similar to the outdoor air as the AER is increased (Weschler and Shields, 2000). Indeed, Meadow (2013) found that the indoor biome reflects the outdoor airborne biome when a building is naturally ventilated. However in the current study, the range of AERs observed in the air conditioned retail stores are lower than those found in naturally ventilated spaces and thus the impact of the AER on the indoor biome may not be as significant. Also, the air exchange rate (AER) in the current study was measured immediately prior to installing the HVAC filter at the beginning of each sampling event. Thus, the determined air exchange rate represents a single time point prior to the microbial tests. The median AER measured at the retail sites was 0.62 air changes per hour (Q_1 0.40/h, Q_3 0.87/h). The small range of AER in the retail facilities may also have played a role in the limited associations to the bacterial communities (Supplemental Table 1). Along with parameters such as indoor relative humidity and temperature, AER can be temporally variable which makes it difficult to correlate these parameters to the month-long averaged indoor microbiome recovered from the HVAC filter dust. Even when only the outdoor environment is considered, bacterial communities in outdoor air have been shown to be highly variable both spatially and temporally (Fang et al. , 2007; Bowers et al., 2011; Bowers et al. , 2013). However, several researchers have found some associations with ventilation strategies and the indoor bacterial communities detected (Meadow et al., 2013; Kembel et al., 2012; Hospodsky et al., 2012).

Conclusions

The indoor environment in retail stores may offer a variety of niches for microbial populations that support a diverse community as seen when compared to other built environment studies. Nevertheless, the most dominant genera observed in the present study have been reported in other indoor environments. The microbiome was significantly influenced by several parameters including human microbiota (most notably to oral and skin bacterial communities) and the outdoor environment. A direct link to human microbial communities has been observed in other indoor spaces, but none with such a low occupant density as the large retail stores investigated in this study. It is possible that even with the low occupant density in the retail stores, the presence of humans still has an impact on the microbial community structure. The other main influence on the indoor retail microbiome was outdoor air. Proteobacteria species were identified in an average of 55% of the sequences, possibly from outdoors; however, only 4 of 23 sites were shown to be significantly influenced by outdoor air through a comparison with other outdoor molecular studies that examine outdoor air utilizing molecular biology-based methods.

The present study found only tangential relationships between the bacterial community present and factors such as season, store location, and store type. However, this study was limited in scope and generalized conclusions may not be extendable for all building types and locations. The air exchange rate was also found not to influence the microbial community. Increasing the air exchange rate, a common approach for reducing indoor pollutants, may not influence the indoor bacterial community in retail stores nor alter the diversity of microbes in the air. Overall, the diversity observed and the lack of clear associations suggest it is advisable to conduct multiple long-term sampling events to adequately characterize the indoor bacterial microbiome of a retail store.

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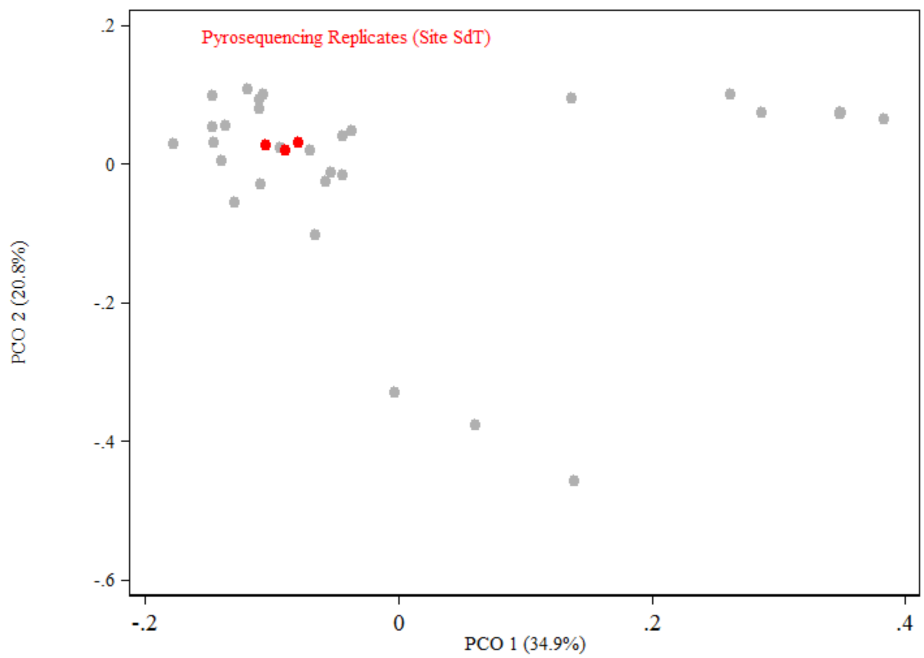
Supplemental Information

Pyrosequencing Reproducibility

Aliquots from the same DNA extract collected from a retail HVAC filter sample were sent for replicate analysis via pyrosequencing. The microbial communities were statistically correlated and clustered together on a PCoA plot (Supplemental Figure 1).

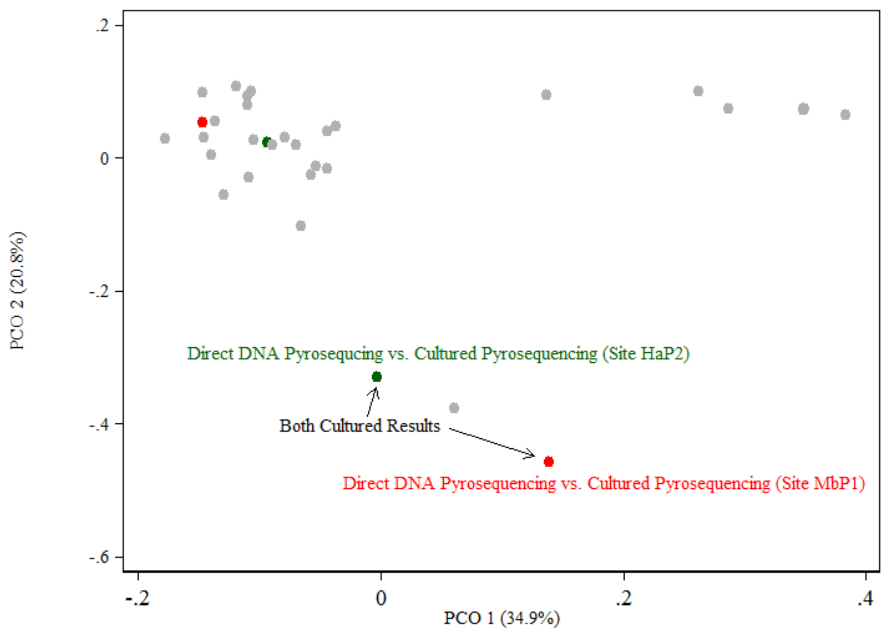


Supplemental Figure 1. Community comparison of pyrosequencing reproducibility of DNA recovered from HVAC filter dust (weighted UniFrac, rarefaction 600 sequences/sample). The red dots represent pyrosequencing replicates. Grey dots represent other retail HVAC samples.

Cultured Community vs. Direct Pyrosequencing

At sites MbP1 and HaP2, the HVAC filter dust was cultured on R2A agar plates containing 0.04% cycloheximide (an anti-fungal agent). After 4 days at 35°C, colonies from the agar plates were removed, the DNA extracted, and processed via pyrosequencing to identify the culturable microbial community. Site MbP1 yielded 11 times fewer operational taxonomic units when cultured as compared to direct pyrosequencing of the DNA extracts (548 vs. 48) and site

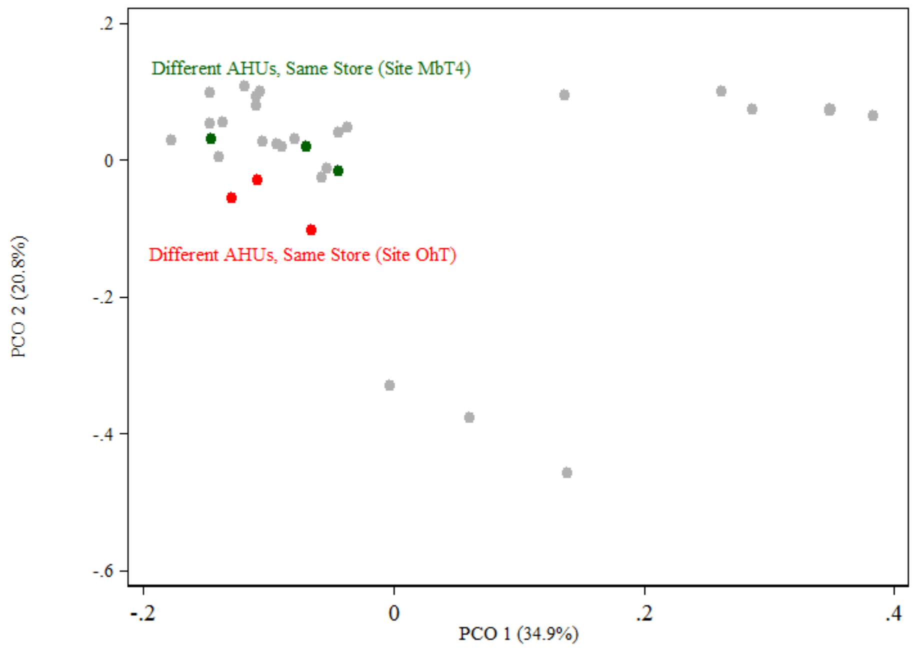
HaP2 had almost four times fewer OTUs when cultured (401 vs. 102). Taxonomic identification showed that the cultures recovered from the agar plates were dominated by the Firmicutes phylum (99% MbP1 and 81% HaP2 sequences), primarily *Bacillales*. In contrast, the direct pyrosequenced results showed a mixture of Proteobacteria, Firmicutes, and Actinobacteria. As shown in the Supplemental Figure 2, the two communities recovered were not the same. A combined analysis of the microbial sequences confirms the limited diversity and bias in cultured results. This result is similar to that reported in other research (Rintala et al. 2008; Zhou et al. 2010).



Supplemental Figure 2. Community comparison of cultured vs. direct pyrosequencing of HVAC filter dust (weighted UniFrac, rarefaction 600 sequences/sample). The red dots represent sampling at retail site MbP1 and the green dots represent sampling at retail site HaP2. Grey dots represent other retail HVAC samples.

Impact of Air Handling Unit

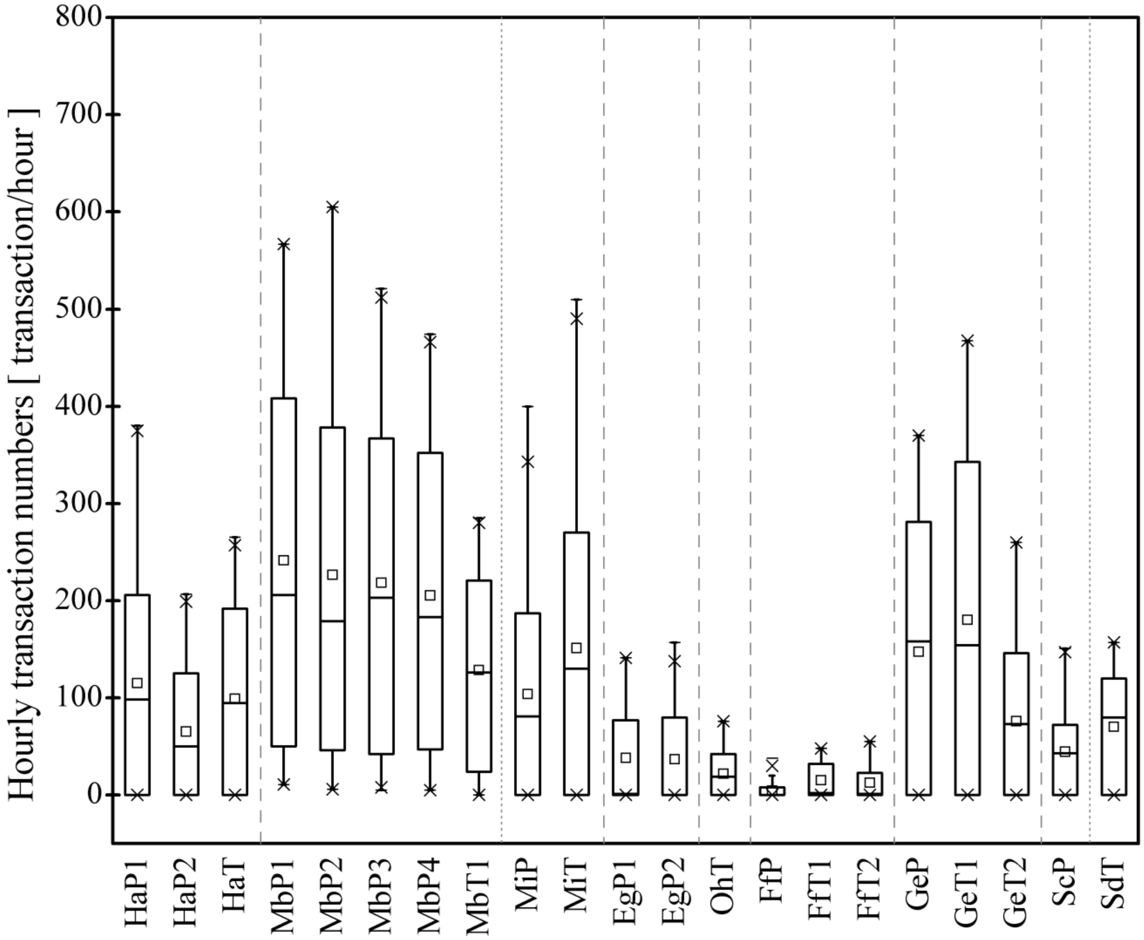
The number of air handling units on the roofs of the retail sites ranged from 2 - 44 independent units per store. At two sites, OhT and MbT4, clean HVAC filters were installed in three rooftop air handling units located across the store. At site OhT, a LEED certified facility; filters were installed at the front (AHU 2), middle (AHU 3), and back (AHU 4) of the store. The number of unique bacterial and fungal OTUs recovered from these filter dust samples varied (Bacterial OTU 61-262, Fungal OTU 116-243). Interestingly, AHU 3 had the greatest number of bacterial OTUs and the least number of fungal OTUs. The relative abundance of bacteria at the phylum level was similar in all three filter samples and statistically correlated ($p < 0.012$). The fungal relative abundance at the class level was also statistically correlated ($p < 0.001$). At site MbT4, filters were installed at the back (AHU 18 east side, AHU29 west side) and front (AHU 20). Like OhT, the number of OTUs were non-uniform across the filters. AHU 20 had the highest number of OTUs at 308, followed by 280 OTUs at AHU 18 and only 61 OTUs at AHU 10. Again, similar to OhT, the relative abundance at the phylum level was significantly correlated ($P < 0.0048$) but the filters did not share many of the most abundant genera. A possible explanation for the differences could be that the overall run time of the units. Measurements of when the air handling units were in operation were taken two days prior to the installation of the filters. For site OhT, the number of OTUs decreased with an increased run time. The opposite effect was observed for site MbT4.



Supplemental Figure 3. Community comparison between different AHUs of HVAC filter dust (weighted UniFrac, rarefaction 600 sequences/sample). Red dots represent retail site OhT at three different AHUs. Green dots represent retail site MbT4 results at three different AHUs . Grey dots represent other retail HVAC samples.

Density in Stores

The density of occupants in retail stores was calculated based on transaction numbers per hour, as reported by the stores over 2-16 days near the time of testing. A generic multiplication factor of 2.2 was applied across all stores to convert transaction number to occupants (shoppers and workers). Yuill (1996) investigated infiltration of automatic doors in retail and other building sectors and observed on average, 2.2 people enter a store each time an automatic door opens. This result does not necessarily preclude the party size to 2.2 people as multiple shoppers may enter on one door opening. Thus, this most likely represents an underestimation of the total shoppers. However, not all shoppers will buy goods plus this factor accounts for workers. The conversion from transaction number to occupants is proprietary information not made available by the retail stores. The conversion depends upon factors including but not limited to conversion rate (shoppers buying/total shoppers) and average time in store. To verify the calculated occupant density was within feasible ranges, the rates were compared to occupancy values reported in the retail categories sales and supermarkets of a U.S ventilation standard (ASHRAE62.1 2010). The results of occupant density in this study range from 0.5-12 individuals/100 m^2, in general agreement with sales (15 people/100 m^2) and supermarkets (8 people/100 m^2). Supplemental Figure 5 shows the hourly transaction numbers with mean (horizontal line), average (small box), large box (25 and 75 percentile), crosses (1% and 99%), and whiskers (highest and lowest values). Overall, the transaction numbers did not vary by season when comparing sites with multiple visits. For the same store, the trends are repeatable on a daily basis with typical inflation on the weekends.



Supplemental Figure 4. The hourly transaction numbers collected from each site

Statistical Analysis

Summary tables of the statistical associations for all building or environmental factors and biological measurements are shown in supplemental Table 1. The significant associations ($p < 0.05$) observed are highlighted in gray. Statistical tests for the analysis included ANOVA, MANOVA, ADONIS, and Spearman Rank Coefficient.

Supplemental Table 1. Summary of Statistical Tests and Associations

	Building Parameters				Environmental Parameters	
	Store Type	**AER**	**Volume**	**Occupant Density**	**Location**	**Season**
Taxa - Phylum	MANOVA p = 0.809	MANOVA p = 0.543	MANOVA p = 0.750	MANOVA p = 0.709	MANOVA p = 0.709	MANOVA p = 0.440
Taxa - Genus	MANOVA p = 0.741	MANOVA p = 0.0624	MANOVA p = 0.995	MANOVA p = 0.227	MANOVA p = 0.473	**MANOVA** **p = 0.0467**
OTU Count	ANOVA R^2 = 0.323 p = 0.363	Spearman Rank rho = -0.179 p = 0.477	Spearman Rank rho = 0.176 p = 0.448	Spearman Rank rho = -0.193 p = 0.441	ANOVA R^2 = 0.009 p = 0.679	ANOVA R^2 = 0.342 p = 0.052
Community Comparison	ADONIS R^2 = 0.320 p = 0.789	ADONIS R^2 = 0.031 p = 0.630	ADONIS R^2 = 0.023 p = 0.795	ADONIS R^2 = 0.076 p = 0.169	ADONIS R^2 = 0.0369 p = 0.518	**ADONIS** **R^2 = 0.264** **p = 0.001**

Additional Site Information

Site specific information is given below and presented in additional detail in the ASHRAE RP-1596 report (Siegel et al., 2013). All the retail sites were standalone buildings unless otherwise noted.

Test sites EgP1 and EgP2 was a large electronics stores in State College, PA. Dust was removed from a pleated filtrite 1” thick, 20” x 20” wire backed HVAC filter (3M, St. Paul MN). This site is located on a frontage road of a major highway. At site EgP2, additional microbial testing included processing DNA extracted using the same protocols as developed for pyrosequencing and analyzed by qPCR 36-panel assay at an EPA certified laboratory.

Test site FfP was a furniture store in Pittsburg, PA. The site is the same brand as the sites FfT1 and FfT2. The filters were pleated and 2” thick, 20” x 20” backed by wire mesh (Flanders, Washington NC). This site was observed to be highly pressurized and the outdoor air vents were not closable, including AHU #1 which contained the filters.

Test site FfT1 and FfT2 was a furniture store on a frontage road along a major highway in Austin, Texas. The installed filters replaced reusable fiber filters normally in place in the air handling units. The tested filters were 2” thick, 20” x 20” (Air Handler, Dallas TX). This site was the same brand as FfP. Like FfP, the AHU tested (AHU 2) did not allow the outdoor damper to be closed.

Test site GeP was a grocery store in Pittsburg, PA. Site GeP was the same brand as GeT1 and GeT2. The 1" thick, 20" x 20" filter (Flanders, Washington NC) was installed in AHU 3.

Test sites GeT1 and GeT2 were conducted in a grocery store in Austin Texas. This site was the same brand as GeP. This site was the only store that shared a wall with another retail space in the present study. The installed filters replaced existing reusable fiber filters. The filters were 2" thick, 24" x 24" (Air Handler, Dallas TX) and placed in AHU 4. At sample site GeT2, the initial filters installed for the study were removed by maintenance contractors.

Test site HaP1 and HaP2 were a home improvement store in State College, PA. This site is the same brand as HaT. Site HaP1 used 2" thick, 24" x 24" filters (American Air Filter, Louisville KY) from AHU 12. Site HaP2 used a pleated 1" thick, 16" x 25" filter (Flanders, Washington NC) installed in AHU 11. This site is located near the intersection of two major highways.

Test site HaT was a home improvement store on the frontage road for a major highway in Austin, TX. This site was the same brand as HaP1 and HaP2. Filters were installed in AHU 11 and consisted of 2" thick, 20" x 20" wire reinforced filters (Air Handler, Dallas TX).

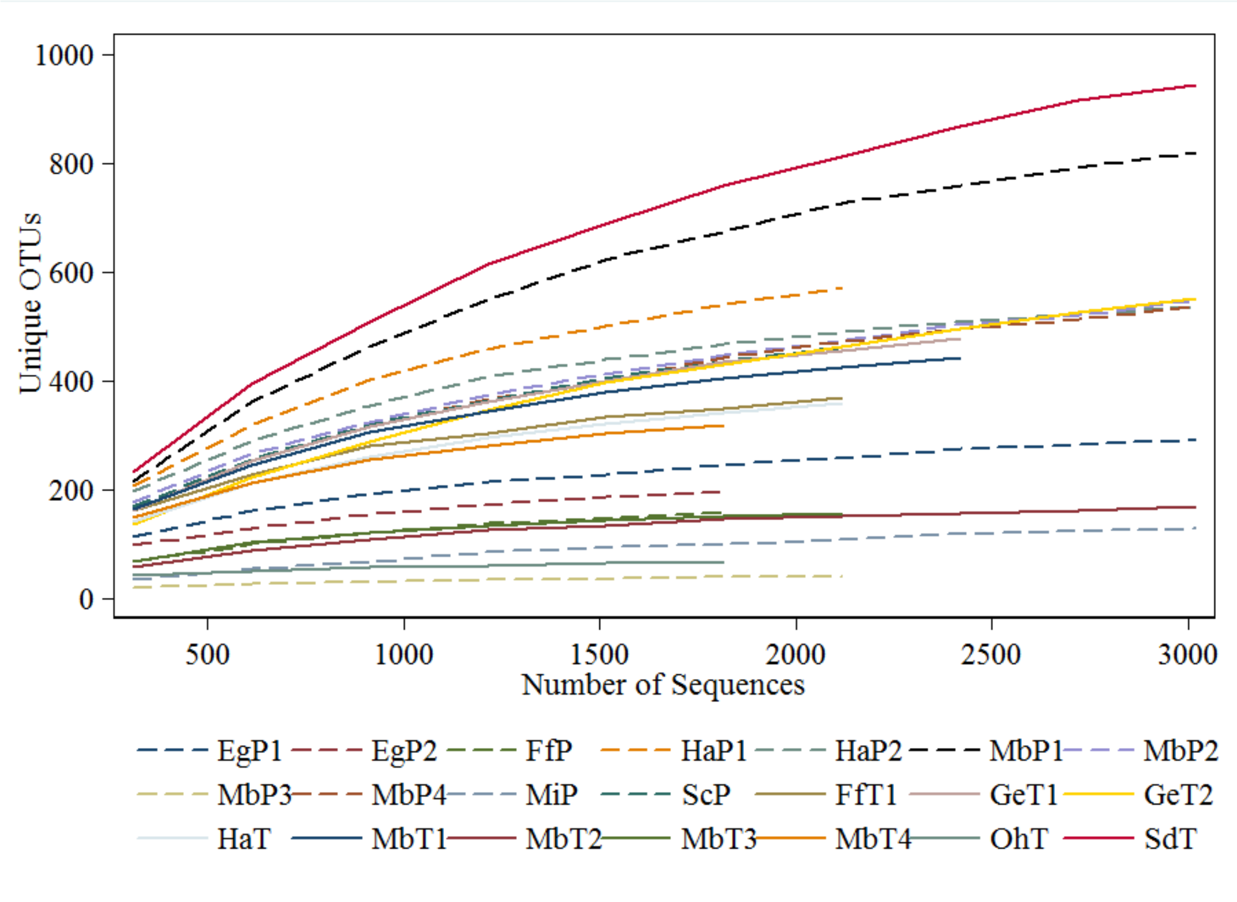
Test sites MbP1, MbP2, MbP3, MbP4 was a general merchandise store in State College, PA. This site was the largest building in this study and the same brand as MbT. The filters were pleated 1" thick, 16" x 20" (Flanders, Washington NC). Filters for MbP1 and MbP2 were installed in AHU 25. Filters for MbP3 and MbP4 were installed in AHU 35. The AHUs are located in close proximity to each other, approximately 100 feet apart. Additional biological testing at site MbP1 included three replicate samples processed from the same filter for a qPCR-based 36-panel fungal assay.

Test sites MbT1, MbT2, MbT3, MbT4 was a general merchandise store in Austin, Texas. This site was the same brand as MbP1, however it was considerably smaller. Filters were installed in AHU 18 and consisted of 2" thick, 20" x 20" wire reinforced filters (Air Handler, Dallas TX). Additional biological tests included Sanger sequencing (MbT1), qPCR 36-fungi panel assay with extraction methods in this study (MbT3), and extracting filter dust from three different AHU to assess variability in a store (MbT4, AHU 10, AHU 18, AHU 20).

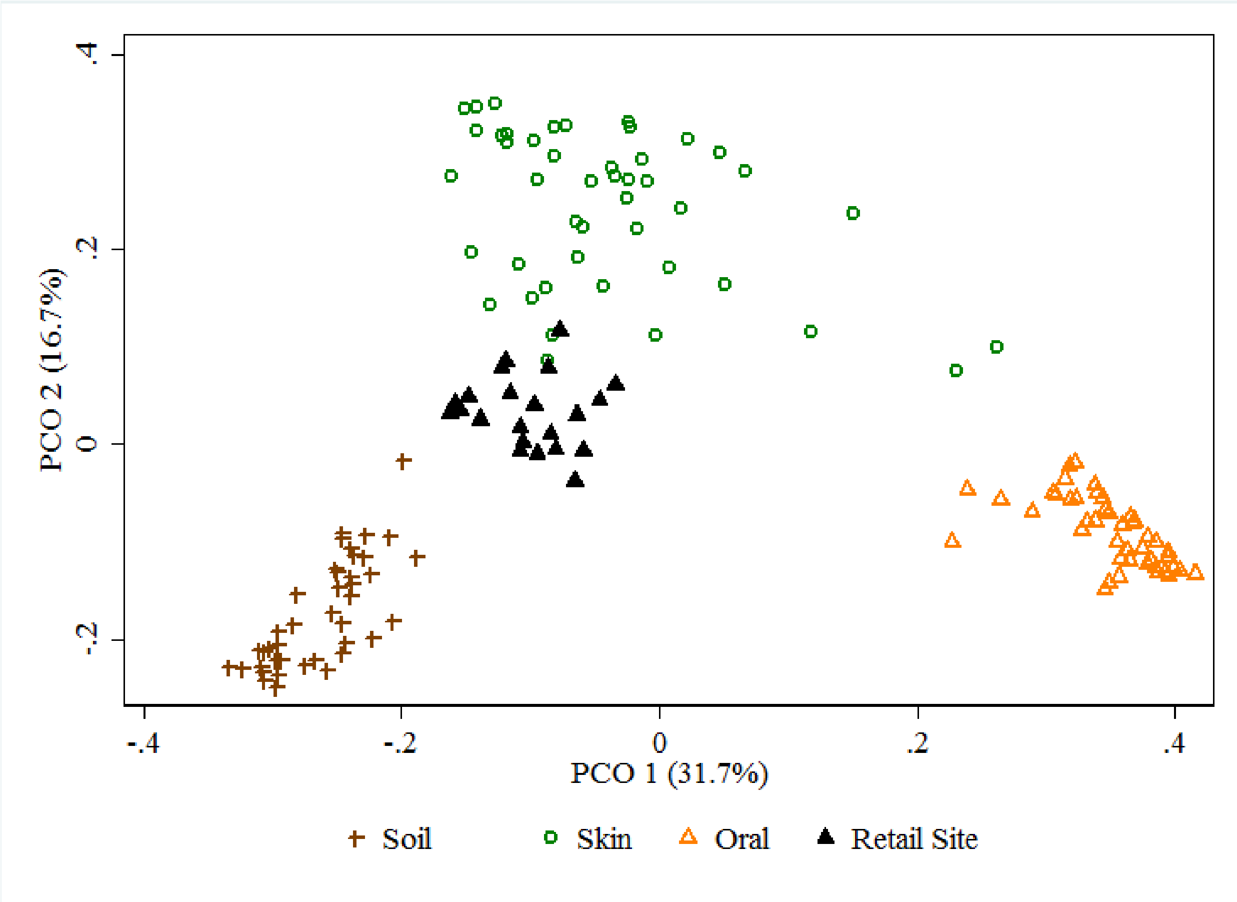
Test site OhT was an office supply store in Austin, TX. The filter were installed in AHU 4 and consisted of 2” thick, 16” x 25” wire reinforced filters (Air Handler, Dallas TX). Additional testing at this store included analyzing filter dust from two other air handling units (AHU 2 and AHU 3). AHU 2 was located near the front of the store and AHU 3 was in the middle of the store.

Test site ScP was a small grocery store located on a major downtown street in State College, PA. The site only contained two air handling units thus the dampers were not able to be closed. The filters installed were pleated 1” thick, 20” x 25” (3M, St. Paul MN).

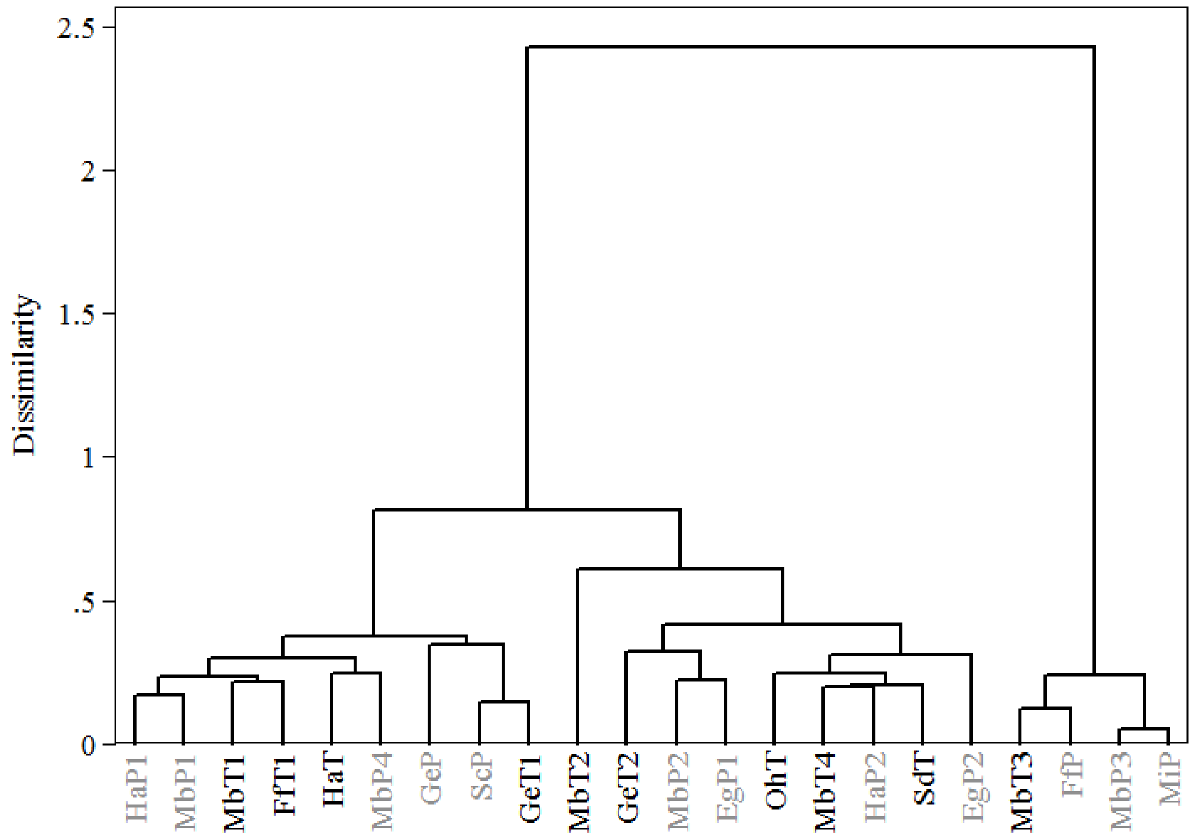
Test site ScT was at a small grocery store located in a dense urban area in Austin, TX. Like ScP, this site contained only two air handling units so the outdoor air dampers were not closed. The filters installed were 2” thick, 16” x 25” wire reinforced filters (Air Handler, Dallas TX). Additional biological tests at site ScT included Sanger sequencing and triplicate runs of the same extracted DNA using pyrosequencing.



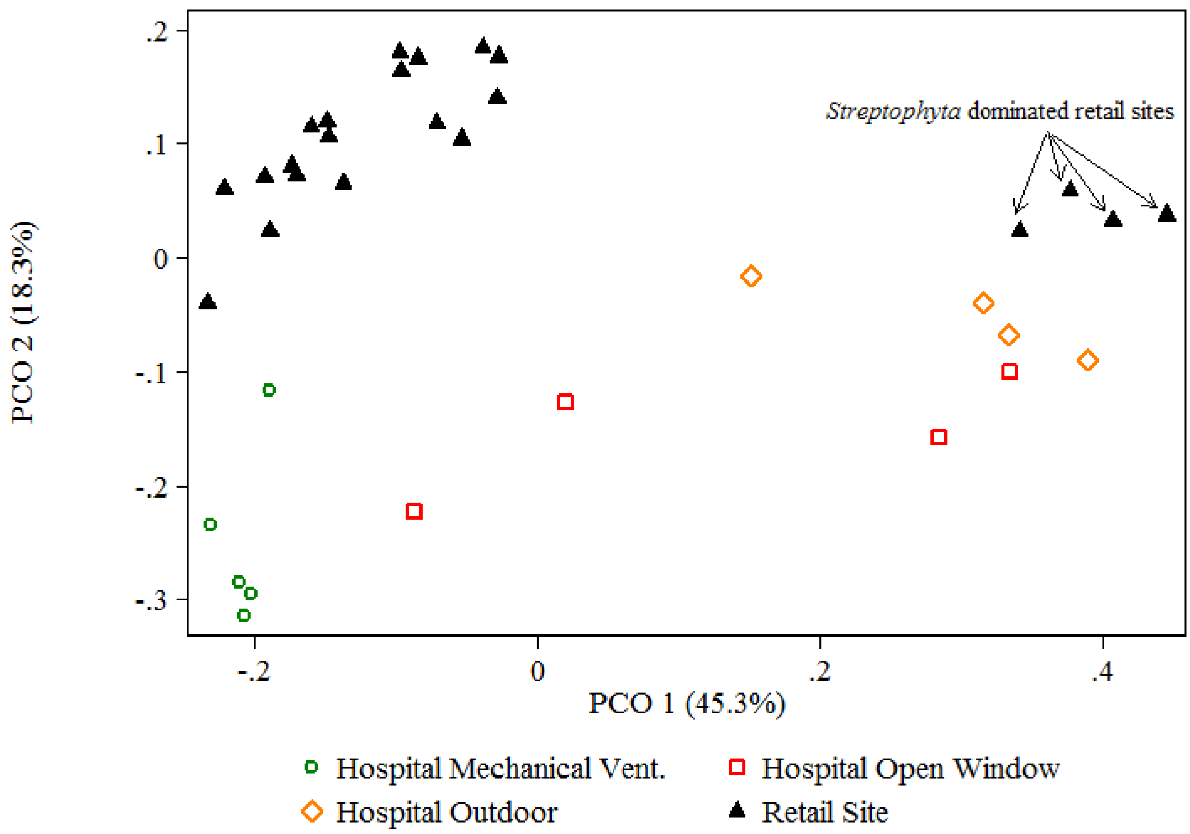
Supplemental Figure 5. Rarefaction Curve for Retail Sites (Dashed Lines for PA, Solid Lines for TX)



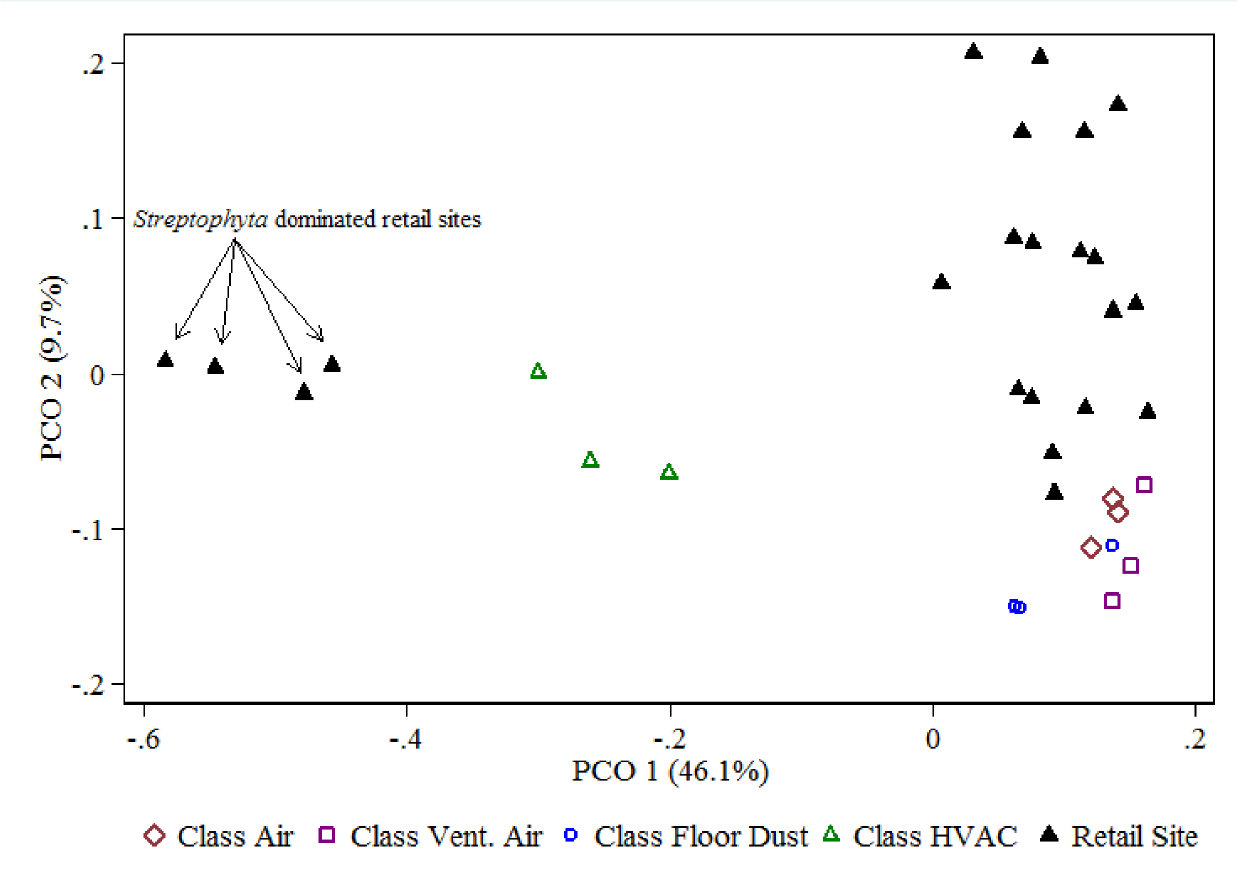
Supplemental Figure 6. Community comparison between retail site, human (from (Costello et al. 2009)), and soil microbiomes (from (Lauber et al. 2009)), Figure 2 from article with outlying gut samples removed (weighted UniFrac, rarefaction 600 sequences/sample)



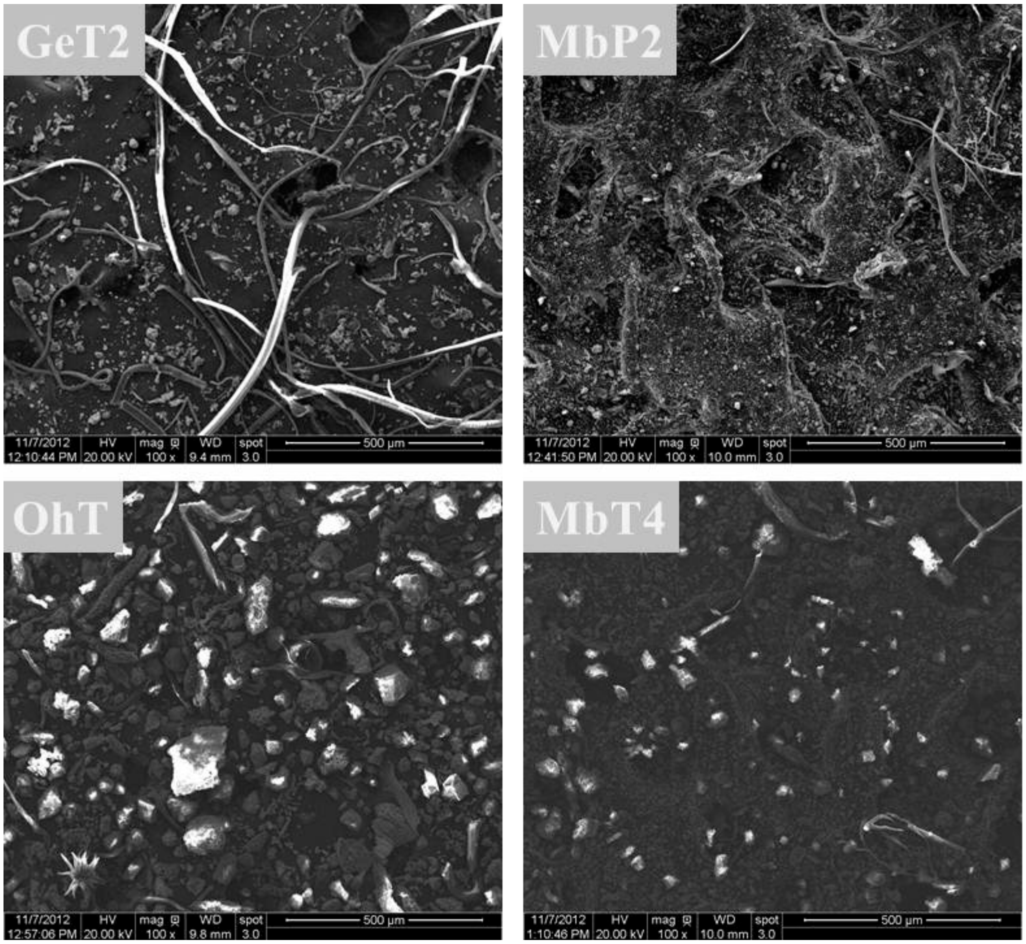
Supplemental Figure 7. Dendrogram clustering of similar microbial communities in retail sites, gray labels from PA, black labels from TX, store type indicated by first letter; E=electronics, F=furniture, G = medium grocery, H = home improvement, M= general merchandise, O = office supply, S = small grocery (weighted UniFrac, clustering wards-linkage, rarefaction 1,200 sequences/sample).



Supplemental Figure 8. Community comparison between retail site and hospital microbiomes (from (Kembel et al., 2012)) (weighted UniFrac, rarefaction 1,200 sequences/sample).



Supplemental Figure 9. Community comparison between retail site and classroom site microbiomes (from Hospodsky et al. 2012) (weighted UniFrac, rarefaction 230 sequences/sample)



Supplemental Figure 10. Scanning Electron Microscope Images from Four Retail Site Dust Samples

Supplemental Table 2. Top Five Most Abundant Genera Detected at Retail Sites

	1	2	3	4	5
EgP1	*Corynebacterium*	*Methylobacterium*	*Pedobacter*	*Propionibacterium*	*Patulibacter*
EgP2	*Methylobacterium*	*Sulfobacillus*	*Eubacterium*	*Corynebacterium*	*Sphingomonas*
FfP	*Methylobacterium*	*Pseudomonas*	*Merismopedia*	*Ralstonia*	*Corynebacterium*
FfT1	*Acinetobacter*	*Bacillus*	*Pseudomonas*	*Brevibacillus*	*Massilia*
GeP	*Acinetobacter*	*Pelagibius*	*Sphingomonas*	*Thauera*	*Pseudomonas*
GeT1	*Hyphomicrobium*	*Acinetobacter*	*Pseudomonas*	*Methylobacterium*	*Sphingomonas*
GeT2	*Chryseobacterium*	*Methylobacterium*	*Corynebacterium*	*Sphingomonas*	*Paracoccus*
HaP1	*Methylobacterium*	*Pseudomonas*	*Acinetobacter*	*Massilia*	*Corynebacterium*
HaP2	*Methylobacterium*	*Corynebacterium*	*Propionibacterium*	*Sphingomonas*	*Micrococcus*
HaT	*Pseudomonas*	*Acidovorax*	*Methylobacterium*	*Brevundimonas*	*Sphingomonas*
MbP1	*Methylobacterium*	*Corynebacterium*	*Pseudomonas*	*Staphylococcus*	*Sphingomonas*
MbP2	*Methylobacterium*	*Corynebacterium*	*Nocardioides*	*Micrococcus*	*Staphylococcus*
MbP3	*Methylobacterium*	*Sphingobium*	*Beijerinckia*	*Flavisolibacter*	*Flavisolibacter*
MbP4	*Acinetobacter*	*Methylobacterium*	*Corynebacterium*	*Paracoccus*	*Pseudomonas*
MbT1	*Corynebacterium*	*Pseudomonas*	*Thauera*	*Staphylococcus*	*Acinetobacter*
MbT2	*Bacillus*	*Paenibacillus*	*Lysinibacillus*	*Acetobacter*	*Corynebacterium*
MbT3	*Methylobacterium*	*Bacillus*	*Sphingomonas*	*Methylocella*	*Clostridium*
MbT4	*Corynebacterium*	*Methylobacterium*	*Staphylococcus*	*Staphylococcus*	*Rubellimicrobium*
MiP	*Methylobacterium*	*Sphingobium*	*Pseudomonas*	*Sphingomonas*	*Herbaspirillum*
OhT	*Pseudomonas*	*Bacillus*	*Sphingomonas*	*Nocardioides*	*Blastococcus*
ScP	*Hyphomicrobium*	*Acinetobacter*	*Corynebacterium*	*Pseudomonas*	*Rubellimicrobium*
SdT	*Methylobacterium*	*Bacillus*	*Sphingomonas*	*Rubellimicrobium*	*Xanthomonas*

Supplemental Table 3. Relative abundance of bacteria in PA retail sites (genera shown with over 2% abundance at one or more sites)

Phlyum	Class	Order	Family	Genus	EgP1	EgP2	FfP	GeP	HaP1	HaP2	MbP1	MbP2	MbP3	MbP4	MiP	ScP
Actinobacteria	*Actinobacteria (class)*	*Actinomycetales*	*Brevibacteriaceae*	*Brevibacterium*	0.0	0.2	0.0	0.2	3.1	0.6	0.8	2.3	0.0	0.1	0.1	0.4
			Corynebacteriaceae	*Corynebacterium*	13.3	4.9	1.3	2.2	3.2	3.6	4.9	7.3	0.0	6.0	0.0	6.8
			Geodermatophilaceae	*Modestobacter*	2.4	0.2	0.0	0.0	0.2	0.9	0.2	0.9	0.0	0.7	0.1	0.1
			Microbacteriaceae	*Microbacterium*	0.0	0.4	0.0	1.4	1.5	2.4	1.9	0.6	0.0	0.0	0.0	0.5
			Micrococcaceae	*Arthrobacter*	0.0	0.0	0.0	0.0	2.3	1.4	0.3	0.2	0.0	0.1	0.0	1.2
				Micrococcus	0.0	0.2	0.0	3.6	2.8	3.3	2.6	4.1	0.0	1.1	0.1	1.2
			Nocardiaceae	*Nocardioides*	1.8	0.6	0.0	0.2	1.5	2.4	0.7	5.8	0.0	0.3	0.2	0.6
			Propionibacteriaceae	*Propionibacterium*	5.1	2.3	0.2	0.3	1.3	3.5	1.6	0.9	0.0	0.8	0.1	0.5
		Solirubrobacterales	*Patulibacteraceae*	*Patulibacter*	4.4	0.3	0.0	0.0	0.7	2.1	1.4	3.4	0.0	0.1	0.0	0.4
			Solirubrobacteraceae	*Solirubrobacter*	2.6	0.0	0.0	0.5	0.2	1.7	0.4	0.8	0.0	0.0	0.1	0.1
Bacteroidetes	*Sphingobacteria*	*Sphingobacteriales*	*Sphingobacteriaceae*	*Pedobacter*	5.7	0.0	0.3	0.0	0.6	0.6	0.5	0.4	0.0	0.8	0.1	0.0
				Sphingobacterium	4.4	0.5	0.0	0.6	0.4	0.0	0.3	0.0	0.0	0.7	0.1	0.1
Firmicutes	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	0.9	3.6	0.9	0.8	1.4	2.7	1.0	1.6	0.0	1.3	0.2	0.5
			Staphylococcaceae	*Staphylococcus*	1.9	0.8	0.2	0.2	1.3	1.2	4.3	3.6	0.0	0.6	0.0	2.0
		Lactobacillales	*Aerococcaceae*	*Aerococcus*	0.0	0.1	0.0	0.0	0.0	1.2	0.3	2.2	0.0	0.2	0.0	0.8
	Clostridia	*Clostridiales*	*Clostridiaceae*	*Clostridium*	1.5	0.4	0.2	0.2	1.2	2.0	0.9	2.3	0.0	0.9	0.1	0.2
			Clostridiaceae unclassified	*Finegoldia*	0.0	1.5	0.0	0.0	0.5	0.0	0.5	2.1	0.0	0.3	0.0	0.1
				Sulfobacillus	2.0	16.8	0.0	0.9	0.4	0.0	0.0	0.0	0.0	0.1	0.0	0.1
			Eubacteriaceae	*Eubacterium*	0.5	6.3	0.3	0.5	0.7	0.0	0.9	1.1	0.0	0.0	0.0	0.3
Proteobacteria	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Brevundimonas*	1.8	1.0	0.9	1.4	1.2	0.9	0.9	0.5	0.0	0.7	0.0	3.3
		Rhizobiales	*Hyphomicrobiaceae*	*Hyphomicrobium*	0.1	0.0	0.0	0.0	0.0	0.0	0.7	0.1	0.0	0.2	0.0	15.2
			Methylobacteriaceae	*Methylobacterium*	8.3	17.6	71.2	0.1	5.9	8.9	8.6	9.5	94.5	6.5	84.1	2.1
		Rhodobacterales	*Rhodobacteraceae*	*Paracoccus*	0.0	0.2	0.1	1.9	2.7	0.4	1.1	1.0	0.0	4.2	0.0	0.6
				Pelagibius	0.0	0.0	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
				Rubellimicrobium	0.1	1.0	0.0	2.0	2.0	0.7	0.7	0.1	0.0	0.9	0.0	5.0
		Rhodospirillales	*Acetobacteraceae*	*Roseomonas*	0.0	1.4	0.1	0.6	0.3	2.0	0.6	0.3	0.0	2.1	0.2	0.6
		Sphingomonadales	*Sphingomonadaceae*	*Sphingobium*	0.0	0.8	0.2	0.5	0.5	0.9	0.3	0.1	4.5	0.2	5.0	0.0
				Sphingomonas	1.4	4.3	1.3	7.0	2.6	3.4	3.6	0.9	0.0	2.2	0.9	2.8
	Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Ralstonia*	1.3	1.6	1.3	3.2	0.1	0.0	0.0	0.3	0.0	0.3	0.1	0.0
			Comamonadaceae	*Acidovorax*	0.5	3.7	0.1	0.1	0.3	0.5	0.6	0.2	0.0	0.1	0.0	1.5
				Variovorax	1.3	2.1	0.1	0.1	0.2	0.1	1.4	0.3	0.0	0.2	0.0	0.5
			Oxalobacteraceae	*Massilia*	0.1	0.0	0.0	0.0	3.2	1.0	2.4	0.1	0.0	1.9	0.1	0.6
				Naxibacter	0.0	0.1	0.0	0.0	0.2	0.0	0.1	0.0	0.0	2.7	0.0	0.0
		Rhodocyclales	*Rhodocyclaceae*	*Thauera*	0.0	0.0	0.0	6.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	Gammaproteobacteria	*Enterobacteriales*	*Enterobacteriaceae*	*Citrobacter*	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
				Enterobacter	0.0	0.0	0.1	1.9	2.7	0.2	1.6	0.1	0.0	0.2	0.1	3.7
				Erwinia	0.0	0.1	0.1	0.4	0.3	0.0	2.0	0.1	0.0	0.0	0.4	0.6
				Pantoea	0.0	0.2	0.0	3.6	0.2	0.0	0.7	0.1	0.0	0.4	0.1	0.3
		Pseudomonadales	*Moraxellaceae*	*Acinetobacter*	0.9	0.0	0.4	19.6	4.5	2.8	3.0	0.5	0.0	15.3	0.1	8.0
			Pseudomonadaceae	*Pseudomonas*	3.2	1.0	8.8	4.5	5.4	2.1	4.5	0.9	0.0	4.0	1.0	6.7
		Oceanospirillales	*Alcanivoraceae*	*Alcanivorax*	0.0	0.0	0.0	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Supplemental Table 4. Relative abundance of bacteria in TX retail sites (genera shown with over 2% abundance at one or more sites)

Phlyum	Class	Order	Family	Genus	FfT1	GeT1	GeT2	HaT	MbT1	MbT2	MbT3	MbT4	OhT	SdT
Actinobacteria	*Actinobacteria (class)*	*Actinomycetales*	*Brevibacteriaceae*	*Brevibacterium*	0.1	1.1	1.8	0.9	2.3	0.2	0.2	0.7	0.9	0.9
			Corynebacteriaceae	*Corynebacterium*	2.0	1.9	13.9	1.0	10.3	1.7	0.8	13.3	1.4	1.0
			Geodermatophilaceae	*Blastococcus*	0.0	0.1	0.0	0.6	0.1	0.0	0.0	0.7	4.7	0.4
			Micrococcaceae	*Kocuria*	0.2	2.0	0.5	1.2	1.4	1.6	0.0	0.0	0.0	0.2
				Micrococcus	1.5	0.5	0.4	0.5	4.5	0.1	0.0	3.4	0.2	0.1
			Nocardioidaceae	*Nocardioides*	0.3	0.3	0.7	0.8	0.1	0.1	0.0	0.3	5.9	1.4
			Propionibacteriaceae	*Propionibacterium*	2.3	0.3	2.2	1.5	1.2	0.0	0.0	1.5	2.7	0.1
			Streptomycetaceae	*Streptomyces*	0.9	0.5	0.0	2.1	0.0	0.8	0.0	0.0	0.7	0.6
Bacteroidetes	*Flavobacteria*	*Flavobacteriales*	*Flavobacteriaceae*	*Chryseobacterium*	1.2	1.2	22.1	0.0	0.2	0.1	0.4	0.0	0.0	0.0
Firmicutes	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	8.9	2.6	0.3	2.7	4.2	71.6	5.5	1.2	9.2	5.5
				Geobacillus	2.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0
				Lysinibacillus	0.0	0.0	0.0	0.0	0.1	2.7	0.0	0.0	0.0	0.0
			Paenibacillaceae	*Brevibacillus*	3.0	0.0	0.0	0.1	0.1	0.6	0.0	0.4	0.0	0.1
				Paenibacillus	0.9	0.3	0.0	1.2	0.2	3.4	0.0	0.1	0.0	0.1
			Planococcaceae	*Planococcus*	0.4	0.0	0.0	0.1	0.0	0.0	0.5	0.3	3.7	0.2
			Staphylococcaceae	*Staphylococcus*	1.7	0.4	1.1	1.1	5.3	0.4	0.1	4.7	0.4	1.0
		Lactobacillales	*Aerococcaceae*	*Aerococcus*	0.3	0.2	3.2	0.0	0.1	0.0	0.4	0.0	0.0	0.1
	Clostridia	*Clostridiales*	*Eubacteriaceae*	*Eubacterium*	0.0	0.0	0.1	0.0	1.5	0.1	0.0	2.1	1.1	0.3
			Ruminococcaceae	*Faecalibacterium*	0.4	0.0	0.1	0.0	0.4	0.0	0.0	0.7	2.0	0.0
Proteobacteria	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Brevundimonas*	0.1	1.1	1.4	3.3	0.6	0.1	0.1	1.1	0.3	0.6
				Caulobacter	0.1	0.4	0.0	0.5	0.4	0.0	0.1	2.2	0.1	1.8
		Rhizobiales	*Hyphomicrobiaceae*	*Hyphomicrobium*	0.5	19.2	0.4	0.0	0.4	0.0	0.0	0.0	0.0	0.1
			Methylobacteriaceae	*Methylobacterium*	2.3	5.6	18.8	5.2	0.5	1.0	74.9	10.8	1.8	9.0
			Rhizobiaceae	*Rhizobium*	2.2	0.5	0.5	1.0	2.7	0.3	0.2	1.9	0.2	0.6
		Rhodobacterales	*Rhodobacteraceae*	*Paracoccus*	0.4	2.2	3.7	0.8	1.3	0.3	0.0	0.7	0.9	1.6
			Rhodobacteraceae	*Rubellimicrobium*	1.5	0.6	0.1	1.0	0.3	0.0	0.2	3.6	1.9	3.3
		Rhodospirillales	*Acetobacteraceae*	*Acetobacter*	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0
		Sphingomonadales	*Sphingomonadaceae*	*Novosphingobium*	0.9	0.5	0.7	0.1	2.5	0.0	0.7	1.8	1.9	0.6
				Sphingomonas	1.9	3.9	4.2	2.8	2.7	0.1	1.8	4.7	8.6	4.3
	Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	*Acidovorax*	0.1	3.0	0.2	6.6	1.1	0.0	0.0	0.1	1.0	0.0
			Oxalobacteraceae	*Massilia*	2.7	0.5	0.2	0.8	2.6	0.2	0.3	0.0	0.0	1.8
		Rhodocyclales	*Rhodocyclaceae*	*Thauera*	0.0	0.5	0.0	0.0	5.4	0.0	0.0	0.0	0.0	0.0
	Gammaproteobacteria	*Enterobacteriales*	*Enterobacteriaceae*	*Enterobacter*	2.0	2.3	0.3	0.6	1.7	0.2	0.1	0.0	0.2	0.5
			Enterobacteriaceae	*Pantoea*	0.8	0.1	0.1	0.2	1.8	0.1	0.4	0.8	2.2	0.4
		Pseudomonadales	*Moraxellaceae*	*Acinetobacter*	9.6	11.4	0.7	1.9	5.0	0.5	0.1	2.4	0.3	1.1
			Pseudomonadaceae	*Pseudomonas*	4.7	5.7	1.0	24.1	5.6	0.1	1.0	2.1	10.9	2.0
		Xanthomonadales	*Xanthomonadaceae*	*Xanthomonas*	0.0	0.9	0.2	0.0	1.2	0.0	0.0	1.1	0.7	2.3

Supplemental Table 5. Median relative abundance of selected human indictor taxa (Dunn et al. (2013)) recovered from retail sites

	Indicator Taxa	Median Relative Abundance
Human Skin	Corynebacteriaceae	4.4
	Propionibacteriaceae	1.8
	Staphylococcaceae	1.4
Human Oral Cavity	Actinomycetaceae	0.0
	Campylobacteraceae	0.0
	Fusobacteriaceae	0.1
	Leptospiraceae	0.0
	Neisseriaceae	0.0
	Pasteurellaceae	0.0
	Prevotellaceae	0.2
	Veillonellaceae	0.2
Human Stool	Bacteroidaceae	0.3
	Lachnospiraceae	0.2
	Rickettsiaceae	0.0
	Ruminococcaceae	0.8

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